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USE OF HEXANE FOR THE QUANTITATIVE RECOVERY OF ORGANOPHOSPHORUS PESTICIDES FROM AQUEOUS SOLUTION

A FIELD METHOD AND A PRESERVATION TECHNIQUE

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SUMMARY

A general method for extraction, preservation and analysis of aqueous solutions containing traces of organophosphorus pesticides has been developed. Samples of water (natural and distilled) were extracted by high-speed stirring after addition of a small volume of hexane to the sample. Extracts of lower concentration were concentrated by evaporation prior to analysis by flame photometric gas chromatography to yield a detection limit of 1 ppb in most instances. Extraction by this method has been successful for 13 of the 16 organophosphorus pesticides used in this study with an efficiency of better than 95% under the experimental conditions described. The extracts from all aqueous solutions were found to be relatively stable for 45 days. Recent developments in the preservation techniques are compared with ours and the advantages of all techniques are discussed.

INTRODUCTION

Organophosphorus pesticides have found increasing use in many aspects of pest control, as the trend to replace organochlorine pesticides is now almost completed. In North America, even with very tight controls, numerous organophosphorus pesticides are already in substantial use, especially in agriculture. The increase in their usage has led to numerous methods of extraction and analysis to monitor their behaviour in the environment. Adequate procedures for the extraction of organophosphorus pesticides, as well as a workable preservation technique ,are essential for the accurate quantitative determination of pesticides in environmental samples. The methods and techniques developed vary widely with the kind of sample to be extracted.

Since the chemical and physical properties of organophosphorus pesticides are relatively similar, several attempts have been made to generalize the method of extraction and the technique of preservation. As early as 1969, a study was made to develop a comprehensive scheme for the extraction of organophosphorus pesticides from river waters and sewage effluents using chloroform as the extractant[1]. Attempts to improve the extraction from waters were made using preconcentration at low temperature[2]. Studies were also performed to develop general methods for the extraction of

organophosphorus pesticides from fruits, vegetables, plants and grains[3–5]. Similar studies, although more specific, have also been reported recently, on the extraction of organophosphorus pesticides from animal tissues[6], fish[7], soils and sediments[5,7–10].

It has generally been accepted that the solvent and the technique used to extract the organophosphorus pesticides are determined by the nature of the material to be analysed. It has been reported, in earlier literature, that materials with a high water content (*e.g.* fruits and vegetables) are blended with polar solvents such as acetonitrile or ethyl acetate[11,12], acetone or methanol[13]. For materials containing both fat and water (*e.g.* milk and meat), mixtures of polar and non-polar solvents, such as methanol–acetonitrile[14] and acetone–methylene chloride[15] have been used. Non-polar solvents such as hexane and benzene have been extensively used for material with high fat content [16].

Preservation of the environmental samples for labotatory analysis has also been the subject of extensive research. The first techniques experimented with were preconcentration and freezing of the samples[17]. A more recent study with several pesticides reported the use of refrigeration, buffers and addition of chloroform to preserve water samples[18]. Resins, specifically Amberlite XAD-2, have also been used for the extraction and preservation of fenitrothion[19] and also for paraquat and malathion[20]. Absorption of organophosphorus pesticides by minerals has been suggested as a preservation technique in a recent study involving parathion and attapulgite[21]. A method for the extraction of chlorpyrifos-methyl from aqueous solution has been developed in our laboratory[22]. We have shown not only that the method is simple enough to be used in the field but also that the chlorpyrifos-methyl extracted from different aqueous solution was preserved in hexane for up to 45 days. Since the use of an organic solvent for the general extraction and preservation technique of organophosphorus pesticides has not yet been reported, we wish to report the use of hexane as an extractant and as a preservation solvent for several organophosphorus pesticides as described below.

EXPERIMENTAL

Preparation and extraction

The pesticides used in this study were analytical grade of the highest purity available (>97%) and were used as received from the manufacturers. Acetone and hexane were pesticide grade and used without further purification. A solution of 1000 ng/μl of each pesticide in acetone was prepared as a primary standard. By addition of proper volumes of the primary standard to measured volume of natural or distilled water, test solutions were made ranging in concentration from 1 ng/μl to 0.001 ng/μl.

These solutions of all pesticides, made with natural or distilled water, were extracted by a modification of our recently reported method[22]. 10 ml of hexane were added to a glass-stoppered volumetric flask containing 300 ml or 1000 ml of solution to which 1 ml of concentrated HCl had been added. The mixture was stirred rapidly for at least 15 min with an electric magnetic stirrer. After separation of the two phases, 5 ml of the hexane layer was recovered for analysis. The samples were dried with Na_2SO_4 and analysed by the gas chromatographic procedure given below. When necessary, extracts containing lower concentrations of the pesticides were evaporated to smaller volume prior to their injection into the gas chromatograph.

Gas chromatographic analysis

A Varian Model 3700 gas chromatograph equipped with a flame photometric detector was operated with an interference filter for spectral isolation of phosphorus emission at 526 nm. The 60-cm glass column (2 mm I.D.) contained 4% SE 40/6% OV-210 on 60–80 mesh Gas-Chrom Q and was operated isothermally at various temperatures as shown in Table I, after being conditional for 24 h at 275°. Except for the nitrogen (carrier gas) as shown in Table I, the flow-rates of the gases were constant throughout the experiment. The conditions for the analysis of specific pesticides are listed im Table I. With the primary standard solution prepared as described above, secondary standards ranging in concentration from 0.001 to 1.0 ng/μl were prepared by dilution with hexane. The calibration curve for each pesticide was established by plotting peak heights against nanograms injected.

TABLE I

EXPERIMENTAL CONDITIONS FOR GC ANALYSIS

Pesticide	*Temperature (°C)*			*Flow-rate carrier gas (ml/min ± 1)*	*Retention time* (min)*
	Injection	*Detection*	*Column*		
Abate (temefos)	240	240	280	80	3.90
Amidithion	230	230	200	30	1.98
Diazinon	220	220	180	30	1.31
Dursban (chlorpyrifos)	220	220	180	30	2.95
Dowco 214 (chlorpyrifos-methyl)	220	220	180	30	1.98
Ethion	220	220	210	30	2.95
Fenitrothion (folithion)	220	220	190	30	2.95
Guthion (azinphos-methyl)	230	230	220	30	1.96
Iodofenphos	230	230	200	30	2.25
Malathion	220	220	190	30	2.50
Methidathion	230	230	200	30	2.57
Ronnel	220	220	190	30	2.55
Trithion	230	230	200	30	1.58
Vapona (dichlorvos)	160	160	100	30	2.09
Phencapton	240	240	220	30	2.45
Phosphamidon	240	240	220	30	2.75

* Average of 5 measurements.

RESULTS

Extraction from aqueous solutions

Extractions were performed on solutions made with distilled water and with natural water (still and running) from marshes, ponds and creeks of the region*. Some of the solutions made up with natural water needed to be filtered before extraction. The samples fortified with each organophosphorus pesticide at concentrations ranging from 1.0 to 0.001 ng/μl were extracted as described above. For all the solutions, the recovery ranged from 89 to 102% for the majority of the pesticides used, as shown in Table II. There are two exceptions worth noting. First, vapona, amidithion and phosphamidon were not extracted with hexane. Second, the recovery percentage of abate at the 0.001 ng/μl level is somewhat low compared with those of the other pesticides; it was never more than 45%.

* Region: 15-mile radius from the University of Trois-Rivières.

TABLE II

RECOVERY OF PESTICIDES FROM AQUEOUS SOLUTION WITH HEXANE

Pesticide	*Volume extracted (ml)* *	*ng/µl added*	*Recovery (%)* **
Abate	300 or 1000	1, 0, 0.1, 0.01 (0.001)	91–98 (45)
Amidithion	1000	1.0	3.3–4
Diazinon	300 or 1000	1.0, 0.1, 0.01, 0.001	93–97.5
Dursban	300 or 1000	1.0, 0.1, 0.01, 0.001	97–101
Chlorpyrifos-methyl	300 or 1000	1.0, 0.1, 0.01, 0.001	96–101
Ethion	300 or 1000	1.0, 0.1, 0.01, 0.001	98.5–102
Fenitrothion	300 or 1000	1.0, 0.1, 0.01, 0.001	91–99
Guthion	300 or 1000	1.0, 0.1, 0.01, 0.001	88–95.5
Iodafenphos	300 or 1000	1.0, 0.1, 0.01, 0.001	96–99.5
Malathion	300 or 1000	1.0, 0.1, 0.01, 0.001	91–97.5
Methidathion	300 or 1000	1.0, 0.1, 0.01, 0.001	93.5–98
Ronnel	300 or 1000	1.0, 0.1, 0.01, 0.001	96.5–100
Trithion	300 or 1000	1.0, 0.1, 0.01, 0.001	97.5–101.5
Vapona	1000	1.0	11–16
Phencapton	300 or 1000	1.0, 0.1, 0.01, 0.001	87–103
Phosphamidon	1000	1.0	<5

* The results were the same using natural or distilled water.

** The recovery percentages reported here are the lowest and highest values of five extractions at each concentration.

Gas chromatographic analysis

Under the conditions described above, all the pesticides used in this study have a retention time of less than 3 min except abate, which has a retention time of almost 4 min, as shown in Table I. In samples containing low levels of the pesticides, the ex-

TABLE III

STABILITY OF THE EXTRACTS

Pesticide	*Recovery (%)* *				
	0 h	*24 h*	*1 week*	*4 weeks*	*6 weeks*
Abate	96	96.5	95	94	92.5
Amidithion	—	—	—	—	—
Diazinon	93	93	93.5	93.5	90
Dursban	99	97	99.5	98	91
Chlorpyrifos-methyl	98.5	98	97	97.5	90
Ethion	100	100.5	99	99.5	98
Fenitrothion	92	95	93.5	94	92
Guthion	98	97	96	96	89
Iodofenphos	96	98.5	97	98.5	88
Malathion	98	97	96.5	94	91
Methidathion	95	94.5	96.5	95	91.5
Ronnel	100	102	99	98	99.5
Trithion	98	97.5	97.5	97.5	89
Vapona	—	—	—	—	—
Phencapton	89	93	93.5	92	89
Phosphamidon	—	—	—	—	—

* The results shown are an average of five extracts.

tracts were evaporated to a smaller measured volume prior to their injection into the gas chromatograph. The concentrations were measured by comparison of their peak heights with the standard curve obtained with known amounts of the pesticides.

Stability studies

A study of the preservation of the extract was undertaken with the pesticides that could be extracted by our method. Extracts were kept in an air-tight container at room temperature and their stabilities were checked periodically. As can be seen from Table III, the amount recovered did not decrease much for up to 4 weeks in most instances. No difference was observed between samples kept in the dark or in the light. In samples where traces of moisture were present, degradation increased drastically for all pesticides used in this study, without exception.

DISCUSSION

Numerous factors, such as the simplicity of the method of extraction as well as its effectiveness as shown by the recovery percentage, the stability of the extracts for up to 45 days, and the accuracy and the speed of the GC analysis, suggest that the method described herein is effective for the extraction, preservation and analysis of organophosphorus pesticides from aqueous solutions. Furthermore, the use of flame photometric GC with specific phosphorus detector and/or sulphur detector, obviates the need for cleaning up the extracts, making this method very easy to adapt for field use.

The method is not effective for all the organophosphorus pesticides that were studied. Extraction of water samples using hexane was not effective with amidithion, vapona and phosphamidon. When chloroform was used as the extractant the recovery percentage was near 100% for all these three. Chloroform had also shown good results as an extractant for most of the other pesticides used in this study; however, it could not be used effectively for their preservation. In most instances the recovery percentage in chloroform increased with time, suggesting strongly that the extract gradually evaporates, even though all the necessary precautions were taken. In a study where chloroform was used for the preservation of organophosphorus pesticides, it was added and kept in solution under water, thus minimizing loss by evaporation[18]. It is interesting to note the similarity of the structures of amidithion, vapona and phosphamidon, in which the acyl chain does not contain a benzene ring. Their overall structures make them more susceptible to extraction by a polar solvent, such as chloroform, than by a non-polar solvent such as hexane. Malathion and ethion, which lack aromaticity, are extracted with high recovery percentage by both solvents, suggesting that the polarity of the molecule has been somewhat diminished by its structural symmetry making it more susceptible to extraction by a non-polar solvent such as hexane.

Earlier studies had shown that addition of concentrated HCl was necessary to optimize the percentage recovery in aqueous solutions containing traces of abate[7] and chlorpyrifos-methyl[22], so the acid was added for the extraction of all the pesticides used in this study. Extraction without HCl was tried for amidithion, vapona and phosphamidon but the percentage recovery did not improve, showing that concentrated HCl did not have an adverse effect on the extraction of these pesticides. Even though Miles *et al.*[7] have reported recently that concentrations of abate as low as $1.0 \cdot 10^{-5}$ ppm were extractable by a method similar to the one described here, we were unable

to detect concentrations lower than 0.01 ppm of this pesticide. Differences in the chromatographic conditions, such as the temperature, the liquid phases and the flow-rate of the carrier gas, might be responsible for this discrepancy. As achieving a retention time of less than 3 min was one of the prime objectives of this study, we set up the conditions on the chromatography to obtain the maximum precision in the shortest time. Under our conditions 0.01 ng/μl was the minimum concentration of abate that was extractable with a recovery percentage of more than 90%. If a lower concentration of abate is suspected the method described by Miles *et al.*[7] should be used.

Comparison of our preservation technique with others reported recently is interesting. Addition of chloroform to water samples containing traces of pesticides, as reported by Bourne[18], is not only a technique with numerous and tedious steps in the extraction procedure, but recovery percentages are lower for natural water samples compared with our results. Moreover, we had already demonstrated that traces of moisture in the extracts increase the degradation of the organophosphorus pesticides markedly and the recovery percentage decreases rapidly.

The use of Amberlite XAD-2 resins as reported by Mallet and co-workers[19] and Tsunenari[20] is certainly a breakthrough in the search for an adequate technique for the preservation of aqueous samples containing traces of organophosphorus pesticides. The effectiveness of their preservation technique is not in doubt, although the need for studies with other pesticides is obvious in order to generalize it for organophosphorus pesticides. Recovery of the pesticides preserved by this technique and their analysis seem more complicated than our method. We concluded that although all three techniques discussed have advantages for the preservation as well as ease of application in the field, we feel that our method has the overall advantage of having a very simple, fast and precise analysis procedure.

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